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The genetics of three polymorphic larval colour forms of *Chlosyne lacinia* (Lepidoptera, Nymphalidæ)

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1. INTRODUCTION

Chlosyne lacinia (Geyer) is a nymphalid butterfly ranging from the Southwestern United States and thence southward to Argentina (Ehrlich & Ehrlich, 1961). The insect is multibrooded and preferably feeds on sunflower (*Helianthus annuus* L.), although high populations have also been observed to be maintained on cocklebur (*Xanthium saccharatum* Wallr.).

The research described in this paper is concerned with determining the inheritance of three polymorphic larval forms. These resemble the nigra, bicolor, and rufa forms reared by Edwards (1893) and first described by Cockerell in 1893 (dos Passos, 1964). The study was initiated in June 1966 from larval types collected in the vicinity of Mesa, Arizona, on sunflower.

2. DESCRIPTION OF LARVAL FORMS

Nigra is a black form, variable in the number of minute white spots dotted over the body surface. Another variable feature is a small yellow ring around the base of each mid-dorsal spine. In some individuals it is almost absent while in others it is very conspicuous.

Bicolor differs from nigra by having a mid-dorsal orange band running the length of the body. This banded appearance is derived from large orange areas that are variable in size and pattern and occur one to each body segment, except the prothoracic and anal segment.

The last form, rufa, is completely orange except for variable amounts of black pigment restricted to the segmented areas of the last instar larva. In early instar larvae this pigment may be diffused between body segments and be present to such an extent that they can easily be mistaken for a bicolor. However, in the last instar the two forms are easily distinguishable.

3. MATERIALS AND METHODS

All rearing was done at 80 °F under an incandescent bulb used as both a heat and light source. The larvae were fed on sunflower plants collected from the field. These were closely inspected for predators and other *C. lacinia* egg clusters and larvae before being used. A mixing of individuals between different progenies was prevented by enclosing each sunflower plant containing an individual progeny with a $\frac{1}{32}$ in. mesh nylon netting.

All sibs of a mating were reared together until the last instar, whereupon a count of larval types was made. Counts at any earlier instar may have led to error due to the close similarity between forms as stated above. After counting, the individuals were segregated according to larval type and allowed to pupate. Upon pupation the pupae were separated individually so that virgin females would be available for mating.

Mating was aided by hand-pairing. The female, after mating, was released in a cage containing sugar water as a food supply and a sunflower terminal upon which egg clusters were oviposited. In most matings each female oviposited more than one egg cluster (up to eight by some females), each containing on the average 200 eggs. Separate larval counts were made of each cluster within a mating but is not enumerated in the data in Table 1 since the egg cluster data did not differ significantly.

4. RESULTS AND DISCUSSION

The data from thirty matings, representing six mating classes (or larval phenotypes of the adults) are given in Table 1. The first column, titled 'mating number', is the label assigned to specific mating pairs belonging to the mating class as given in the second column. Column 3 gives all possible larval genotypes of adults in each mating class in accordance with the proposed genetic mechanism for which progeny data was obtained. The expected larval ratio in the progeny from parents with these genotypes follows next under the heading 'expected progeny ratio'. The next two columns contain the observed progeny data of the thirty matings. The probability values that resulted from chi-square tests based on the inheritance proposed below are in the last column.

The genetic mechanism proposed to explain the observed ratios of the nigra, bicolor, and rufa larval forms is a non-linked, two-locus system with the nigra and bicolor determining alleles at one locus and those of rufa at the other. The dominant allele at the rufa locus, symbolized by the letter *R*, is epistatic, such that the nigra and bicolor phenotypes are expressed only if its recessive homologue, symbolized by the letter *r*, exists as a homozygote. The dominant allele at the nigra–bicolor locus is symbolized by *B* and produces the bicolor phenotype, either as a homozygote or a heterozygote. Its recessive allele, symbolized by *b*, in the homozygous condition gives the nigra form.

The proposed phenotype–genotype relationships are thus as follows: nigra, *bb,rr*; bicolor, *Bb,rr BB,rr*; and rufa, *bb,Rr Bb,Rr BB,Rr bb,RR Bb,RR BB,RR*.

The proposed mechanism is supported, first, by the close agreement of the observed with the expected larval ratios, as is evidenced by the probability values given in the last column of Table 1, and, secondly, by the multiple generation lineage data (Fig. 1).

Matings were assigned to specific parental genotypic classes on the basis of the following observations: (1) The observed offspring phenotypes dictated only one possible parental genotypic class. (2) The observed number of offspring from a mating was sufficient to consider the lack of a phenotype in the offspring as an indication of the lack of expected offspring of such a phenotype. (3) Consideration of the multiple generation lineage data with the above observations dictated only one possible parental genotype.

The first two reasons apply to all matings but 23a, 23b, 23c, 23e, 12a, 26l, 26c, and 26q. A combination of the first and third reasons apply to matings 23a, 23b, 23c, and 23e. In Fig. 1 the parental genotypes are not given since this can be obtained from Table 1.

In the case of matings 12a, 26l, 26c, and 26q, definitive assignment of a specific genotype was not possible on the basis of the above. These matings were therefore tentatively assigned the genotype that best explained the observed offspring ratios. In none of the 30 matings did any offspring data or lineage relationships contradict the proposed model of inheritance.

In summary, the genetic mechanism supported by the data is a non-linked, two locus, epistatic system. The bicolor and rufa determining alleles are dominant over their recessive homologues with the dominant rufa allele epistatic over the nigra–bicolor locus.

Table 1. *Last instar progeny data*

Mating no.	Mating class	Possible parental genotypes	Expected progeny ratio			Total no. of offspring	Total offspring of each phenotype			Minimum probability value associated with the observed and expected ratio
			Nigra	Bicolor	Rufa		Nigra	Bicolor	Rufa	
24d	Nigra × nigra	$bb, rr \times bb, rr$	1	0	0	228	228	—	—	—
27b			1	0	0	316	316	—	—	—
2a	Nigra × bicolor	$bb, rr \times Bb, rr$	1	1	0	485	254	231	—	0·20
15a			1	1	0	123	72	51	—	0·05
15b			1	1	0	332	156	176	—	0·20
15c			1	1	0	112	50	62	—	0·20
251	Bicolor × bicolor	$BB, rr \times B-, rr$*	0	1	0	161	—	161	—	—
10b		$Bb, rr \times Bb, rr$	1	3	0	102	22	80	—	0·05
16a			1	3	0	143	36	107	—	0·95
16b			1	3	0	87	13	74	—	0·030
22b			1	3	0	180	40	140	—	0·20
22c			1	3	0	455	118	337	—	0·50
25i			1	3	0	82	20	62	—	0·80
25j			1	3	0	202	62	140	—	0·05
12a	Bicolor × rufa	$Bb, rr \times Bb, Rr$	1	3	4	857	131	304	422	0·040
21a		$Bb, rr \times BB, Rr$	—	—	—	—	—	—	—	—
		$BB, rr \times —, Rr$†	0	1	1	53	—	26	27	0·80
17b	Rufa × rufa	$B-, Rr \times BB, Rr$	0	1	3	465	—	125	340	0·20
261		$bb, Rr \times Bb, Rr$	1	1	6	121	18	10	93	0·20
26b		$RR \times R-$‡	0	0	1	125	—	—	125	—
26m			0	0	1	160	—	—	160	—
26o			0	0	1	258	—	—	258	—
26p			0	0	1	58	—	—	58	—
26v			0	0	1	123	—	—	123	—
23a		$Bb, Rr \times Bb, Rr$	1	3	12	851	54	155	642	0·80
23b			1	3	12	1166	79	215	872	0·50
23c			1	3	12	264	22	45	197	0·20
23e			1	3	12	206	13	37	156	0·99
26c			1	3	12	175	13	23	139	0·05
26q			1	3	12	84	5	13	66	0·50
28b	Nigra × rufa	$bb, rr \times Bb, Rr$	1	1	2	292	68	73	151	0·50

* $B-$ stands for BB or Bb.

† The state of the B locus is immaterial in the rufa mate.

‡ The state of the B locus is immaterial.

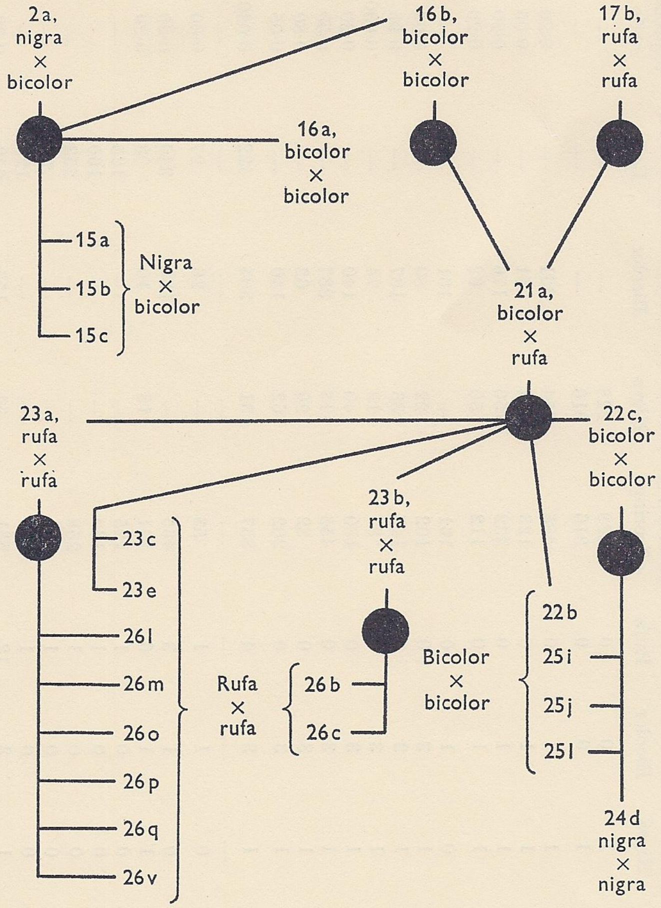

Fig. 1. Multiple generation lineage chart.

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